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(54) Title: BIOSENSORS WHICH UTILIZE CHARGE NEUTRAL CONJUGATED POLYMERS
(54) Titre: BIOCAPTEURS UTILISANT DES POLYMERES CONJUGUES A CHARGE NEUTRE

(57) Abstract

This invention encompasses charge neutral conjugated polymer or copolymers which have a functional group for binding a biomolecule probe; electrodes and array of electrodes in electrical contact with such polymers and wherein a biomolecule probe is covalently lined to the polymer. The invention includes biosensors which utilize the conjugated polymer coated electrodes wherein the binding to the biomolecule probe is detected by electrical means such as AC impedance.

(57) Abrégé

La présente invention concerne un polymère conjugué à charge neutre ou des copolymères qui possèdent un groupe fonctionnel permettant la liaison d'une sonde biomoléculaire. Cette invention concerne aussi des électrodes et un réseau d'électrodes en contact électrique avec ces polymères, et une sonde biomoléculaire est liée de façon covalente au polymère. Cette invention comprend des biocapteurs qui utilisent les électrodes revêtues du polymère conjugué, et la liaison de la sonde biomoléculaire est détectée par une composante électrique telle qu'une impédance relative au courant alternatif.

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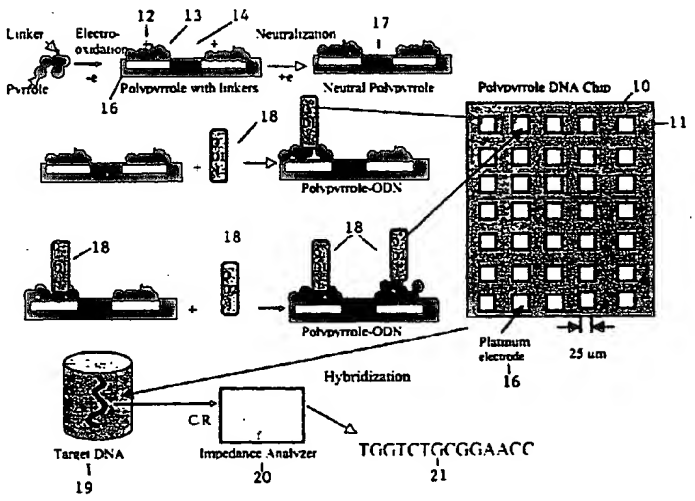
(54) Title: BIOSENSORS WHICH UTILIZE CHARGE NEUTRAL CONJUGATED POLYMERS



(57) Abstract: This invention encompasses charge neutral conjugated polymer or copolymers which have a functional group for binding a biomolecule probe; electrodes and array of electrodes in electrical contact with such polymers and wherein a biomolecule probe is covalently lined to the polymer. The invention includes biosensors which utilize the conjugated polymer coated electrodes wherein the binding to the biomolecule probe is detected by electrical means such as AC impedance.

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Description

BIOSENSORS WHICH UTILIZE CHARGE NEUTRAL CONJUGATED POLYMERS

This invention claims the priority of provisional application Serial No. 60/138,437 filed June 10, 1999.

FIELD OF THE INVENTION

The invention is in the field of arrays of sensing electrodes on a chip for conducting analysis of biological substances such as DNA.

BACKGROUND OF THE INVENTION

A device for biomolecule detection is generally comprised of supporting matrix for probe molecule attachment or entrapment, a sensing probe located on/in the supporting matrix. When exposed to a complementary biomolecule target (or analyte), the biosensing device produces detectable change in radioactive, optical, or electrical signal to confirm the existence of a specific biomolecule target. In general, the biomolecule target to be detected needs to be labeled with a marker (or reporter) such as ^{32}P, fluorescent dye, or redox, depending on whether the detection means is autoradiography, fluorescent microscope or electric tools.

An alternative biosensing device includes a second reporting molecule. The second reporting molecule is introduced after the probe molecule has interacted with its complementary biomolecule target. Like the probe molecule, the second reporting molecule also interacts with the biomolecule target by either binding to the target or forming a complex.

Lavache, et al Analytical Biochemistry 258, 188-194 (1998) describes an

oligonucleotide array constructed on a silicon chip having a matrix of addressable microelectrodes. Each electrode is coated with polypyrrole copolymer where some of the pyrroles in the copolymer have an oligonucleotide bound to the pyrrole. The polymers are made by electrochemical techniques. This copolymer is deposited on microelectrodes. Hepatistis C genotypes were detected by hybridization of the probe DNA on the electrode to test sample DNA which was PCR amplified to contain a fluorescent marker group.

WO 95/29199 describes functionalized polypyrrole copolymers where the functional groups are designed to bind biological molecules such as DNA or polypeptides.

US Patent 5,837,859 assigned to Cis Bio International describes the preparation of electrically conductive pyrrole/nucleotide/derivatized/pyrrole copolymers useful for nucleic acid synthesis, sequencing and hybridization. The copolymers are produced electrochemically and coated on microelectrodes for DNA analysis.

US Patent 5,202,261 describes conductive sensors and their use in diagnostic assays.

US Patent 5,403,451 describes the detecting of a target analyte with conductive

polymer coupled with periodic alternating voltage.

In a typical prior art, the target DNA is usually labeled with a marker (or reporter)

such as ^{32}P, fluorescent dye, or redox. When the labeled target is exposed to its complimentary probe on the conductive polymer or copolymer, a radioactive signal, or fluorescence, or electric signal is detected. Generally, fluorescent or redox labeling

is preferred due to the stringent experiment conditions required for radioactive labeling. However fluorescent dyes in the vicinity of conductive polymers or copolymers are subject to signal quenching. On the other hand, conductive polymers or copolymers contribute to significant background noise when used for redox labeled target detection.

SUMMARY OF THE INVENTION

It is an object of the present invention to eliminate the signal quenching from conductive polymers when used as supporting matrix for probe attachment or entrapment for biomolecule detection and a biosensing device to carry-out such detection.

It is another object of the present invention to reduce the detection noise from conductive polymers when used as supporting matrix for probe attachment or entrapment for biomolecule detection.

It is still another object of the present invention to provide a simplified method for biomolecule detection and a biosensing device to carry-out such detection.

The invention is directed to a method of detecting biological molecule (biomolecule) such as DNA, RNA and polypeptides with the aid of a neutralized conjugated polymer or copolymer on electrodes. Compared to prior art, the present invention makes use of a functionalized polymer or copolymer in its neutral state, instead of conductive state as the supporting matrix for biomolecule probe attachment or entrapment in a biomolecule detection device.

In one embodiment of the invention, aromatic monomers and functionalized aromatic monomers are electrochemically polymerized and deposited on an electrode surface to generate a functionalized polymer or copolymer. The as-deposited conjugated polymer or copolymer is in a charged, conductive state. In present

invention, the charged, functionalized polymer or copolymer is electrochemically reduced to a neutral state to form (charge neutral conjugated polymer) before it is used in any biomolecule detection.

The charge neutral functionalized polymer or copolymer has low electric background when used in electric detection of biomolecules. It also does not quench fluorescent signal when used in fluorescent detection of biomolecules. In both cases, the resulting devices have significantly improved signal to noise ratio, thus enhancing the sensitivity of biomolecule detection.

Thus, the invention includes a charge neutral conjugated polymer which have functional groups for binding biomolecule probes to the polmyer. The invention includes electrodes in electrical communication with such polmyers, arrays of such electrodes. The invention includes biosensors which a biomolecule probe is covalently linked to the functional group of the charge neutral conjugated polymer on electrode and a binding of a biomolecule to be detected is measured by an electrical detection means, such as AC impedence.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents a schematic diagram for preparing the array of polypyrrole coated electrodes and detecting by AC impedance.

Figure 2 illustrates polypyrrole copolymer formulation.

Figure 3 illustrates the electrochemically reduced neutral polypyrrole copolymer.

Figure 4 illustrates the relationship of capacitance vs. frequency on oxidized polypyrrole-based electrodes with and without DNA Attachment.

Figure 5 illustrates the relationship of capacitance vs. frequency on neutral polypyrrole-based electrode with and without DNA attachment.

Figure 6 illustrates the comparison of response of capacitance vs. frequency between oxidized and neutralized polypyrrole-based electrodes with DNA attachment.

Figure 7 AC impedance planes measured in perfect match hybridized DNA and single stranded DNA system.

Figure 8 is a Frequency Complex diagram obtained from neutralized polypyrrole Electrodes.

Figure 9 is impedance planes measured in 3-bas mismatch hybridized DNA and single stranded DNA systems.

Figure 10 is a plot of Resistance vs. $\omega^{-1/2}$ for AC impedance measured in 3-base mismatch hybridized DNA and single stranded DNA systems.

DETAILED DESCRIPTION OF THE INVENTION

The invention is directed to a method of detecting biological molecule with the aid of a charge neutral conjugated polymer on electrodes. Charge neutral conjugated polymer is meant a polymer with zero charge (negative or positive) on its backbone, yet with delocalized pi electron on its backbone. A conjugated polymer is characterized by its backbone with regular alternation of single and double chemical bonds. Examples of conjugated polymers include: polypyrrole, polyphenylene, polyacetylene, polydiacetylene, polythiophene, polyfuran, polyaniline, polycarbazole, poly(phenylene vinylene). More specifically, the invention encompasses a charge neutral conjugated polymers containing one or more functional groups capable of binding a probe molecule. The charge neutral conjugated polymer deposited on the surface of electrodes by electrochemical copolymerization of aromatic monomers and functionalized monomers as is known in the art. The as-deposited conjugated polymer or copolymer is conductive and is usually in its charged state with its charge

being balanced by counter ions from the polymerization solution. The charged state is the source of signal quenching for nearby fluorescent markers as in the case of fluorescence detection. It is also the source of noise for electric detection.

To overcome these potential problems, the polymer or copolymer deposited on the electrode used in present invention is reduced to its charge-neutral state from the as-deposited charged state by reverse biasing right after the polymer or copolymer is initially deposited on the surface electrodes. The polymer or copolymer in its neutral state is an insulator or semiconductor, which does not quench fluorescence of nearby fluorescent markers in fluorescence detection and also give rise to only limited background noise in electric detection of biomolecule target.

The functional group used in present invention includes, but not limited to, amine, hydrazine, ester, amide, carboxylate, halide, hydroxyl, vinyl, vinyl carboxylate, thiol, phosphate, silicon containing organic compounds, and their derivatives. The functional group is used to bind biomolecule probes such as DNA, RNA, peptides, polypeptides, proteins, antibody, antigen and hormones to the polymer or copolymer on the electrode. For example, an oligonucleotide which is in part complementary to a target DNA is covalently linked to a neutral polypyrrol copolymer through an amine functional group.

The electrode used in the present invention is made of at least one of the following materials: metals such as gold, silver, platinum, copper, and alloys; conductive metal oxide such as indium oxide, indium-tin oxide, zinc oxide; other conductive materials such carbon black, conductive epoxy and combinations thereof.

The preferred sensing method in this embodiment is electric or electrochemical methods. After exposure to a target molecule, the biosensor senses a change in electric signal, and reports the change by a readout means such as display,

printout. The electric or/and electrochemical methods may be selected from, but are not limited to, AC impedance, cyclic voltammetry (CV), pulse voltammetry, square wave voltammetry, AC voltammetry (ACV), hydrodynamic modulation voltammetry, potential step method, potentiometric measurements, amperometric measurements, current step method, and combinations thereof.

It is more advantageous to detect a biomolecule target without the need of labeling the target. Present invention provides a highly sensitive method for detection of biomolecule target without the need of labeling the target.

Some biomolecules are electrically active and may produce undesired background noise when a detection is performed by passing charge through those biomolecules. For example, guanine and adenine can be oxidized around 0.75 V and 1.05 V, respectively. (Analtica Chimica Acta 319 (1996) 347-352). Thus it is more desirable to use impedance methods for labelless biomolecule detection.

The invention includes a method for determining an analyte in a test sample comprising:

(a) depositing a polymer or copolymer film on an electrode by electrochemically polymerizing an aromatic monomer and a monomer with functional group in a solution via a positive bias with supporting electrolyte;

(b) neutralizing the polymerized polymer by applying a reverse bias to the electrode;

(c) attaching covalently a biomolecule probe to the neutral copolymer through the functional monomer;

(d) contacting the electrode with the test sample containing an electrolyte; and

(e) measuring the change of electric or electrochemical properties of the electrode by electric or/and electrochemical methods when the analyte is bound to the biomolecule probe on the neutral polymer or copolymer.

The biosensor may include an array of electrodes in electrical contact with a matrix of charge neutral conjugated polymer having different sensing probes for sensing multiple biomolecule targets. It is also within the scope of the present invention to fabricate a high density biosensor with column and row addressable electrodes coated with thousands of sensing probes for screening applications. In the case of a high density array, it is more practical to place various biomolecule probes on each electrode with a robotic tool.

The invention is illustrated by neutral polypyrrole conjugated polymer electrode arrays used in conjunction with AC impedance detecting methods. The process to make such arrays is schematically shown in Fig. 1. The chips 10 are made by microelectric technology on a silicon support 11. The probe arrays 15 and electrodes 16 are made of inert metals such as gold or platinum. Polypyrrole 12, with DNA linking group 13 is electrochemically deposited on the probe array 14 in 0.1 M pyrrole + 5 μM 3-acetate N-hydroxysuaccinimido pyrrole + 0.1 M $LiClO_4$/acetonitrile (0/5% water). Then the polypyrrole-film is electrochemically neutralized 17. Using a nanofluidic-dispensing tool, every probe can be sequentially attached to a different oligonucleotide 18 ODN1 and ODN2. After a hybridization of the probe arrays in a target ODN2 solution 19, AC impedance analyzer 20 is used to detect the impedance change for a specific DNA sequence 21.

In a variation of the aforesaid embodiment, the biomolecule probe can also be attached to the aromatic functional monomer before it is electrochemically polymerized with aromatic monomer to yield a conjugated polymer.

This invention will be further described by the following example with polypyrrole as the conjugated copolymer and DNA as detection target. The example is intended to illustrate specific embodiments of the invention but not to limit this invention in spirit or scope.

Example 1

In order to demonstrate this invention, platinum electrodes with a diameter of 2 mm were used for electrochemical deposition of polypyrrole. The electrode surface was polished by gamma alumina powder (CH Instruments, Inc.) with 0.3 and 0.005 μm in sequence followed by deionized water washing. After polishing, the electrodes were immersed in 1 M H_2SO_4 for 20 minutes and then vigorously washed by DI water. CH 660 potentiostat was used for polypyrrole deposition. Platinum wire and Ag/AgCl were used as the counter electrode and reference electrode, respectively. A solution containing 0.1 M pyrrole + 5 μM 3-acetate N-hydroxysuaccinimido pyrrole + 0.1 M $LiClO_4$/acetonitrile (0.5% water) was prepared as the electrolyte. Cyclic voltammetry (CV) was used for the electrochemical deposition. The potential range for the CV was 0.2-1.3 V vs Ag/AgCl for the first cycle and then changed to –0.1 to 1.0 vs Ag/AgCl for other five cycles. An electrochemical oxidation of the pyrrole produced polypyrrole as shown in Figure 2.

The electrolyte was purged by nitrogen gas during whole electrochemical deposition. The deposited polypyrrole film with the linking function group was uniform and blue in color. The polypyrrole film is in oxidized form (charged conductive state). To make a neutralized polypyrrole, the electrode was placed in the

electrolyte again and cycled over a potential range of –0.2 to 0.3 vs Ag/AgCl, which is the reduction zone for this electrochemical system. The neutralization of the polypyrrole film is illustrated in Figure 3. The neutralized polypyrrole film coated electrodes were vigorously washed for probe oligonucleotide attachment.

Then a 5'-amino-substituted oligonucleotide was attached onto the neutral polypyrrol film by a direct substitution of the leaving N-hydroxysuaccinimide group in dimethylformamide containing 10% phosphate buffer at pH = 8.0 at room temperature for 16 hours. The oligonucleotide CCC TCA AGC AGA with a terminal amino group on it s 5'-phosphorylated position was used. For comparison, the oxidized polypyrrole film was modified by oligonucleotide in the same procedure mentioned above.

Oxidized and neutralized polypyrrole deposited electrodes with and without DNA attachment were tested in deionized water by Solartro 1260 impedance analyzer. A platinum sheet with area of 10 cm^2 was used as the counter electrode. Frequency sweeping method with a bias of 500 mV was conducted over frequency range of 100 mHz to 1 MHz. Since the double layer capacitance is proportional to the area of the electrode surface, the capacitance of the counter electrode surface, $C_c >> C_p$. C_p represents the probe electrode capacitance. Thus, the total capacitance of the detecting system $C_t = 1(1/C_p + 1/C_c) = C_p\, C_c/(C_p + C_c) = C_p$. In addition, the solution resistance for a disc-shaped ultramicroelectrode can be expressed as:

$$R_u = 1/(4kr) \quad (1)$$

Where r is the radius or the side length of the electrode and k is the conductivity of the solution. The R_u contributed from the small probe is much larger than the counter electrode. The results obtained from the AC impedance can

represent the change from the probes, since the surface area of the probe is much smaller than that of the counter electrode.

Experimental results are shown in Fig. 4, 5 and 6. Fig. 4 shows the capacitance changes of the electrode surface vs. frequency, indicating that the oxidized polypyrrole-based electrode surface with oligonucleotide attachment has larger capacitance response than the surface without oligonucleotide attachment at the low frequency range. However, the ratio of signal to noise is not great. Fig. 5 demonstrates that the capacitance of neutralized polypyrrole-based electrode surface with oligonucleotide attachment is significantly greater than that of the surface without oligonucleotide attachment. Fig. 6 shows that the capacitance on the neutralized polypyrrol-based electrode surface with oligonucleotide attachment is greater than that of oxidized polypyrrole-based surface by about 4 times.

The hybridization of the oligonucleotide probe on neutral polypyrrole with its complementary strand shows significant improved signal to noise ratio as compared to that on charged polypyrrole.

Example 2

The neutralized polypyrrole film coated electrodes were vigorously washed for DNA attachment. The electrodes coated with polypyrrole were placed in a mixture of 80 μL of DMF and 20 μL of 15 nM of 5i-amino-3i-fluorescein labeled 15 bp oligonucleotide for 4 hrs at room temperature. At the end the electrode was washed with TBE buffer, deionized water thoroughly, and dried at room temperature in the air. The condition is not optimized.

The 5'- amino-substituted oligonucleotide of 300 uMconcentration in 25 uL of dimethylformamide containing 20% phosphate buffer at pH = 8.0 was attached onto the neutral polypyrrole film on a microelectrode by a direct substitution of the leaving

N-hydroxysuaccinimide group at room temperature for 16 hours. The oligonucleotide CCC TCA AGC AGA with a terminal amino group on its 5'-phosphorylated position was used as an example. After the reaction, the microelectrode was washed with DI water thoroughly before a baseline AC impedance was measured. For hybridization, the probe attached to polypyrrole on a microelectrode was exposed to 35 uL of target molecule of different concentration (μM to aM) in 1x SSC buffer. The hybridization takes place in a sealed conical tube at 37 C in a water bath for 24 to 48 hrs. The microelectrode was then washed with ample amount of 1x SSC solution at room temperature before AC impedance measurement.

A Solartron Impedance Frequency Analyzer 1260 with Electrochemical Interface 1287 was used to measure the impedance before and after hybridization of the polypyrrole microelectrodes. The counter and reference electrodes were platinum and Ag/AgCl, respectively. The measurements were conducted at open circuit voltage (OCV) in 1 M $LiClO_4$ solution. The measured complex impedance versus frequency is shown in Fig. 8 for single and hybridized DNA, indicating significant difference of the impedance before and after hybridization.

In this experiment, this type of electrodes can detect 0.1 amol of target DNA in solution due to the neutralized form of polyrrole film.

Example 3

Experiments for the specificity of the polypyrrole based electrodes were conducted. Eight probes attached electrodes were hybridized in buffers containing 2pM and 2 fM of perfectly matched and three base mismatched target 15mer DNAs, respectively. Results show significant difference between perfect and mismatched

hybridized DNA. Further, the electrodes were placed in 1XSSC buffer for 30 min. of washing at 37 and 38^{0}C, respectively. AC impedance measurements demonstrate that the AC impedance for the mismatched hybridization was getting closer and closer to the baseline of the single stranded DNA with the increase of the washing temperature while that for the perfectly matched hybridization was almost keeping constant. The results are shown in Fig. 7 and 8. Fig. 9 is plotted from Fig. 8, indicating that the resistance in the mismatched DNA system continuously decreases with the increase of the washing temperature going back to the baseline of the single stranded DNA.

This invention can be used in any solution containing metal or polymerized cations, which are ion-conductive and can react with DNA.

The above examples are intended to illustrate the present invention and not to limit it in spirit or scope.

Claims

What is claimed is:

1. A charge neutral conjugated polymer having functional groups for binding a biomolecule probe.

2. A charged neutral conjugated polymer of claim 1 with a functional group for binding a biomolecule probe to the charge neutral conjugated polymer wherein the charge neutral conjugated polymer is selected from the group consisting of polypyrrole, polyphenylene, polyacetylene, polydiacetylene, polythiophene, polyfurna, polyaniline, polycarbazole, poly(phenylene vinylene) and copolymers and combinations thereof.

3. A polymer of claim 1 wherein the polymer is prepared by electrochemical polymerization.

4. A polymer of claim 1, wherein the functional group is selected from the group consisting of amine, hydrazine, ester, amide, carboxylate, halide, hydroxyl, vinyl, vinyl carboxylate, thiol, phosphate and silicon containing organic groups.

5. An electrode in electrical communication with the charge neutral conjugated polymer of Claim 1.

6. An electrode of claim 5 wherein the electrode comprises gold, silver, platinum, copper, and alloys, indium oxide, indium-tin oxide, zinc oxide; or carbon black, conductive epoxy, or combinations thereof.

7. An array of electrodes of Claim 6.

8. The array of electrodes of claim 7 wherein the charge neutral conjugated polymer is polypyrrole.

9. A biosensor device for detecting a biomolecule comprising a an electrode which is in electrical communication with a matrix of charge neutral conjugated polymer having a functional group wherein a biomolecule probe is covalently linked to the functional group and a means for electrically detecting the binding of the biomolecule to the biomolecule probe.

10. A biosensor for detecting a biomolecule comprising an array of electrodes which are in electrical communication with a matrix of charge neutral conjugated polymer having a functional group wherein a biomolecule probe is covalently linked to the functional group and a means for electrically detecting the binding of the biomolecule to the biomolecule probe.

11. The biosensor of claim 10 wherein the electrical detection means is selected from AC impedance, cyclic voltammetry (CV), pulse voltammetry, square wave voltammetry, AC voltammetry (ACV), hydrodynamic modulation voltammetry,

potential step method, potential step method, potentiometric measurements, amperometric measurements, current step method, and combinations thereof.

12. The biosensor of claim 11 wherein the electrical detecting means is AC impedance.

13. The biosensor of claim 12 wherein one or more of the electrodes have an oligonucleotide which is party complementary to a target DNA covalently linked to the charge neutral conjugated polymer in electrical communication with the electrodes.

14. The biosensor of claim 13 wherein DAN in a test sample is hybridized to the oligonucleotide covalently linked to the charge neutral conjugated polymer on one or more of the electrodes in the array.

15. A method for determining an analyte in a test sample comprising:

(a) providing an electrode which is in electrical communication with a matrix of charge neutral conjugated polymer which has covalently linked a binding group which directly or indirectly binds to the analyte;

(b) contacting the matrix of charge neutral conjugated polymer with the test sample containing the analyte; and

(c) electrically detecting the analyte bound to the neutral conjugated polymer.

16. The method of claim 15, wherein the analyte is detected by AC impedance.

17. A method for preparing an analytical electrode comprising:

(a) electrochemically polymerizing pyrrole and a functionalized pyrrole to provide an oxidized polypyrrole functionalized pyrrole copolymer;

(b) electrically depositing the copolymer on an electrode;

(c) electrically reducing the copolymer to provide an electrically neutralized copolymer; and

(d) covalently linking a biomolecule probe that directly or indirectly binds to an analyte to the functionalized pyrrole in the copolymer.

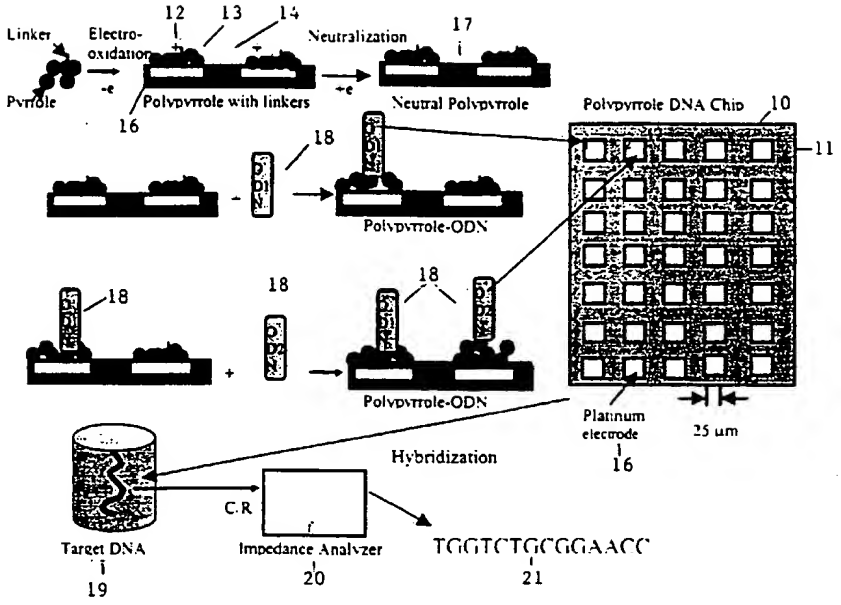
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FIGURE 1

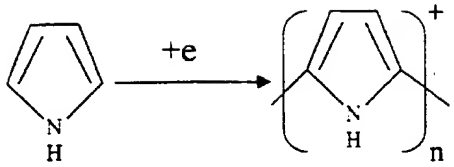

FIGURE 2

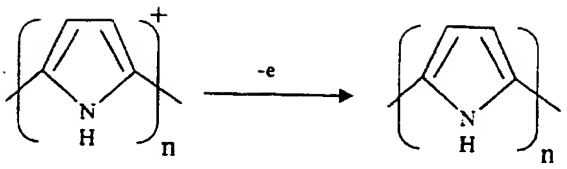

FIGURE 3

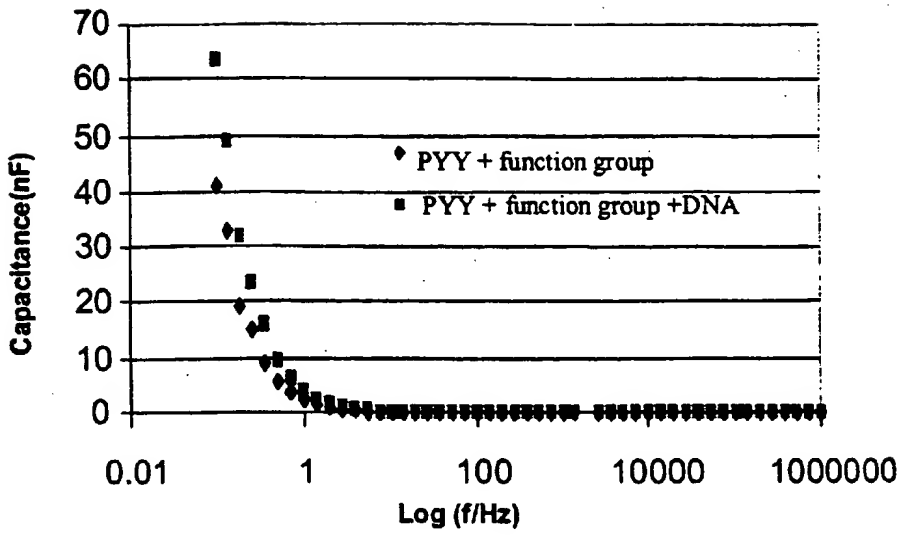
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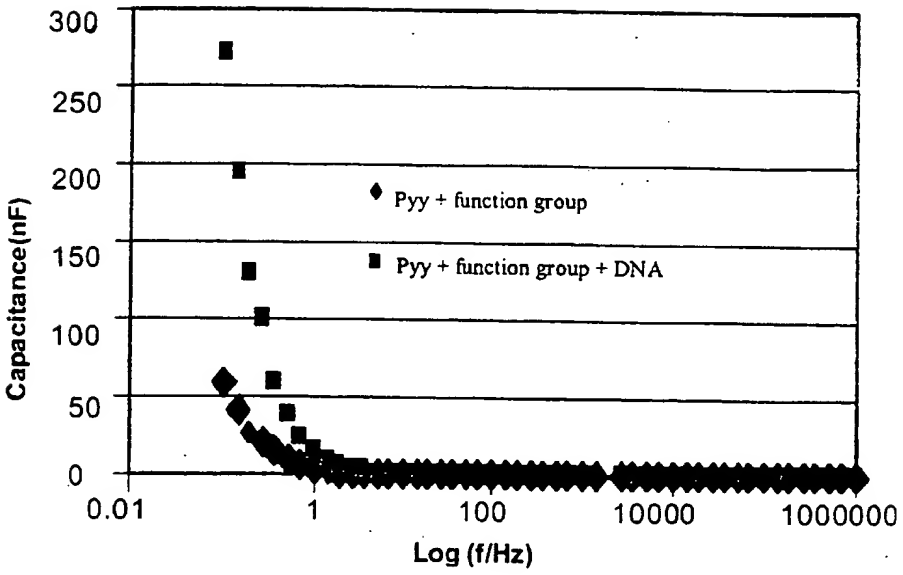
FIGURE 4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Log (f/Hz)

FIGURE 5

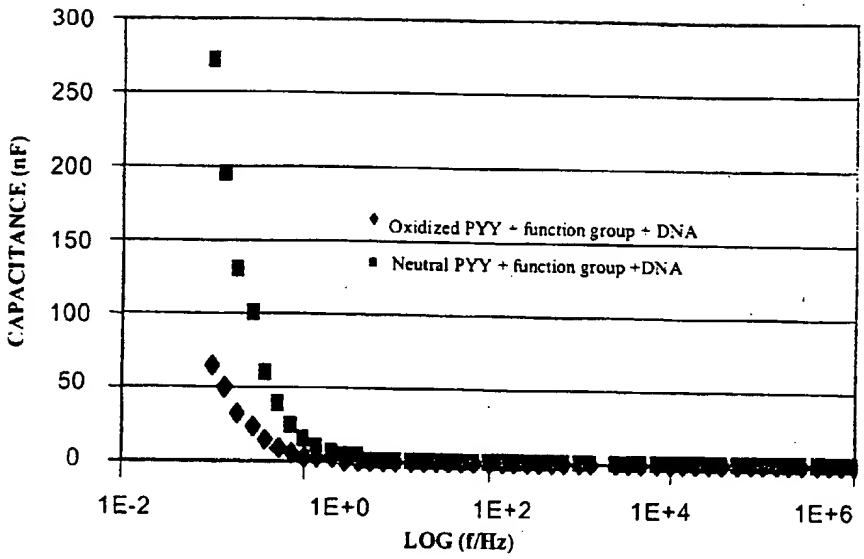
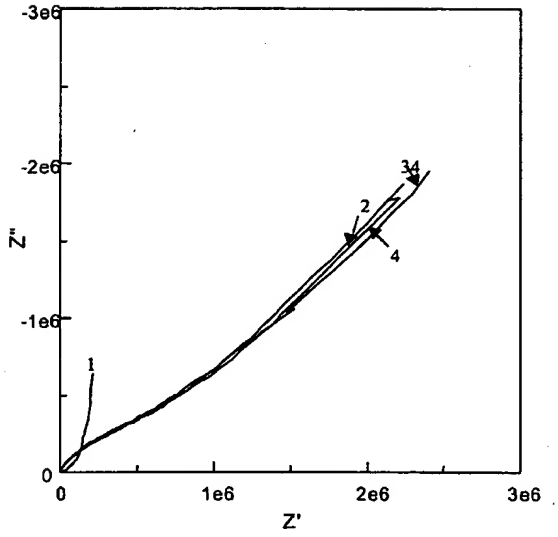

300
250
200
150
100
50
0
CAPACITANCE (nF)
Oxidized PYY + function group + DNA
Neutral PYY + function group +DNA
1E-2
1E+0
1E+2
1E+4
1E+6
LOG (f/Hz)

FIGURE 6



1. Single stranded DNA (Probe)
2. Hybridized DNA with perfect match in 30 µl of 2 pM target for 48 hrs
3. After washing 2 at 37 C for 30 min.
4. After washing 3 at 38 C for 30 min.

Fig. 7 AC impedance planes measured in perfect match hybridized DNA and single stranded DNA system

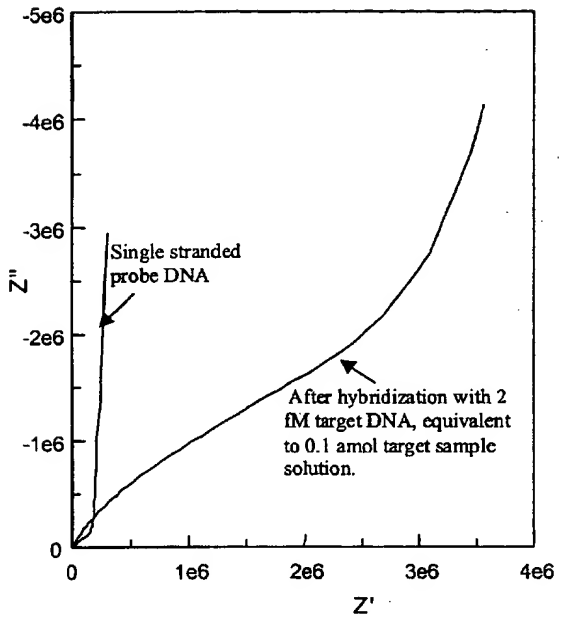
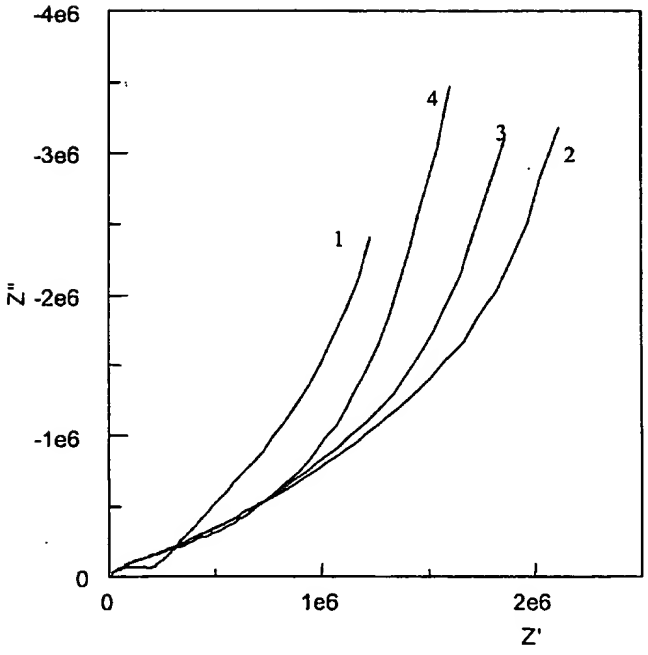
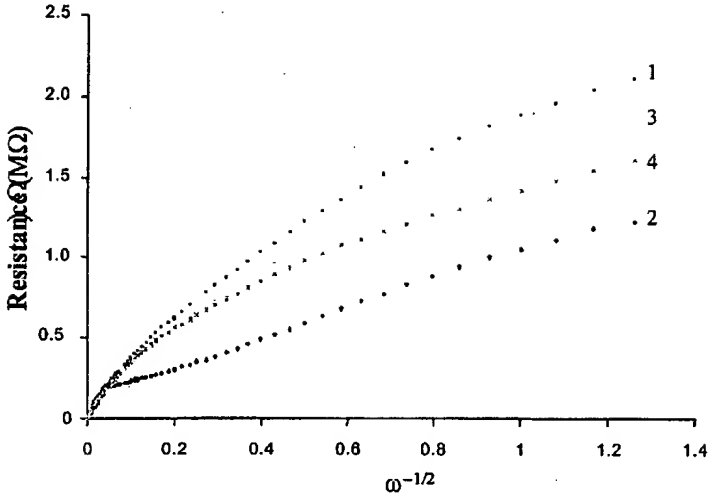


Fig. 8 Frequency Complex diagram obtained from neutrolized polypyrrole Electrodes



1. Single stranded DNA (Probe)
2. Hybridized DNA with 3 mismatch in 30 μl of 2 pM target for 48 hrs
3. After washing 2 at 37 C for 30 min.
4. After washing 3 at 38 C for 30 min.

Fig. 9 AC impedance planes measured in 3-base mismatch hybridized DNA and single stranded DNA systems



1.After hybridization with 2 pM target DNA and wash 1 hour at 25 C
2. Single stranded DNA (Probe)
3. After washing 1 in 1XSSC at 37 C for 1 hour
4. After washing 3 in 1XSSC at 38 C for 1 hour

Fig. 10 Plot of Resistance vs. $\omega^{-1/2}$ for AC Impedance measured in 3-base mismatch hybridized DNA and single stranded DNA systems

INTERNATIONAL SEARCH REPORT

Inte. ional Application No
PCT/US 00/15832

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/543 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	SARGENT, ANITA ET AL: "The electrochemistry of antibody-modified conducting polymer electrodes" J. ELECTROANAL. CHEM. (1999), 470(2), 144-156 , XP000964567 the whole document ---	1-17

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 November 2000	24/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hart-Davis, J

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INTERNATIONAL SEARCH REPORT

Inte. ional Application No
PCT/US 00/15832

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	PREZYNA, LYNETTE A. ET AL: "Interaction of cationic polypeptides with electroactive polypyrrole /poly(styrene sulfonate) and poly(N-methylpyrrole)/poly(styrenesulfonate) films" SYNTH. MET. (1991), 41(3), 979-81 , XP000964589 the whole document	1-6,15, 16
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